invention. Toxins consisting of multiple subunits include, but are not limited to, cholera toxin, pertussis toxin, diphtheria toxin, heat-labile toxin of pathogenic *E. coli*, and canditoxin, etc. The subunit from a toxin, *per se*, generally exhibits adjuvant activity (JP-A Hei 2-243633). Accordingly, toxin of the invention can be such a subunit.

Further, toxins to be utilized as the adjuvant of the invention can be a mutant of the natural toxin. The mutant may have a modified structure in regard to the amino acid sequence, sugar chain and others of the natural toxin. Mutant toxins can be produced, for example, by using artificial mutant strains generated from toxin-producing wild-type or related strains by treating them with mutagenizing agents. Such a toxin is herein referred to as "artificial mutant toxin". The mutant toxin can be also produced by recombinant cells, to which mutant toxin-producing capacity has been given by utilizing gene recombination techniques. The latter is herein referred to as "recombinant mutant toxin".

Because a mutant toxin has a partially altered structure as compared with that of the natural molecule, it generally loses some of the original properties of the toxin as a result of such structural changes. However, there are cases in which the activity of enhancing immunity is retained. When the toxic activity of mutant toxin is sufficiently low, e.g., reduced by a factor of at least one-two thousandth relative to that of the natural one, it can be used as an attenuated toxin for the adjuvant of the present invention provided its activity of enhancing immunity has been verified. Otherwise, the mutant toxin is further attenuated by using any of the above-mentioned chemical and physical treatments to give rise to a usable adjuvant. It is preferable that the mutant toxin is stable under the conditions where the toxin-containing vaccine is to be used. Further, mutants wherein the restoration of toxic activity is limited or negligible are preferable. "Restoration of toxin activity" refers to a phenomenon in which the toxic activity, which has been reduced greatly by a variety of treatments, is recovered as time progresses after the treatment.

There is no particular limitation on regions where the structures are to be changed in an artificial mutant toxin, so long as the specified

three types of amino acid residues remain unchanged and the mutant still has the adjuvant activity. For example, such structural alteration is exemplified by one or more alterations of amino acid residue of oligopeptide moiety, sugar residue of oligosaccharide moiety, organic acid moiety, and such in the toxin molecule.

It can be difficult to artificially choose an amino acid residue or sugar residue to be mutated in the artificial mutant. Therefore, in general, it is difficult to select the type of mutation a priori. However, the reduction of toxic activity and the activity of enhancing immunity are easily verified during research and therefore mutants that meet the requirements of the present invention can be obtained. Further, it has the advantage that mutants with alterations of unexpected amino acid residues can be isolated.

On the other hand, it is possible to choose amino acid residues or sugar residues to be mutated and to intentionally introduce a desired mutation in regard to the recombinant mutant toxin. Specifically, such alterations include one or more alterations of amino acid residue of oligopeptide moiety, sugar residue of oligosaccharide moiety, organic acid moiety, and such in the toxin molecule. The adjuvant of the invention also includes oligopeptide fragments such as peptide fragments playing an important role in the expression of the activity of enhancing immunity.

Production of natural toxin and mutant toxin:

Natural toxins can be obtained as the products of wild-type strains. Alternatively, the toxins can be obtained as the products of a variety of artificial mutant strains such as overproducing strains. Or the toxins can be obtained as the products of recombinant cells, in which the genes of the wild-type strain and such from the same species or a different species, have been introduced by genetic engineering. Further, the production of the toxins can be conducted by procedures of chemical synthesis.

Bacterial toxins utilized in the present invention can be extracted, separated, purified and produced, for example, using a culture of toxin-producing bacterium as a starting material and combining conventional methods. In regard to cholera toxin, an

exemplary method is as follows. Toxin-producing cholera bacterium (*Vibrio chorela*) is cultured at 36°C for 18 hours. The culture liquid is used as a culture seed for large-scale cultivation on agar plates or liquid culture media. After the culture is complete, the culture supernatant (in the case of liquid culture) is concentrated by ammonium sulfate precipitation or ultrafiltration. The toxin is then purified using one or more conventional methods such as column-chromatography using Sephadex and such, ultracentrifugation, gel electrophoresis, etc. The toxic activity in a sample is assayed during cultivation or purification by the procedure described later.

In regard to pertussis toxin, an exemplary production method is as follows. A pertussis bacterium (*Bordetella pertussis*; Tohama; phase I) is cultured in Bordet-Gengou medium to give a culture seed. The seed is transferred into Cohen-Wheeler medium and cultured for 48 hours and the culture supernatant is used as a starting material. The culture supernatant is loaded onto a column of hydroxyapatite. The column is washed with 0.1 M phosphate buffer (pH 7.0), and subsequent elution is performed with 0.1 M phosphate buffer (pH 7.0) containing 0.5 M NaCl. The toxin is further purified by column chromatography, using CM-Sepharose or other carriers on which adequate affinity ligands are immobilized. The toxic activity of a sample is assayed during cultivation or purification by conventional methods.

In addition, toxins produced by mushrooms can be extracted and purified from mushrooms that grow naturally or that are cultivated. A illustrative method for producing animal toxin is as follows: a toxin-producing animal, for example a snake, is bred for the toxin. The toxin is collected from the toxin-producing organ in the mouth. As the matter of course, if commercially available, the toxin may be purchased from the suppliers. Production processes for the toxin and quality thereof can be variously altered as far as the alterations meet the standard for the corresponding biological preparations and are in accordance with related laws and regulations.

An artificial mutant toxin can be produced by an artificial mutant strain derived from toxin-producing wild-stain. The artificial mutant strain is generated from the wild-strain by the treatment with a mutagenizing agent, for example. Known methods of mutagenesis